Supporting Information

A Polymeric Fastener Can Easily Functionalize Liposome Surfaces with Gadolinium for Enhanced Magnetic Resonance Imaging

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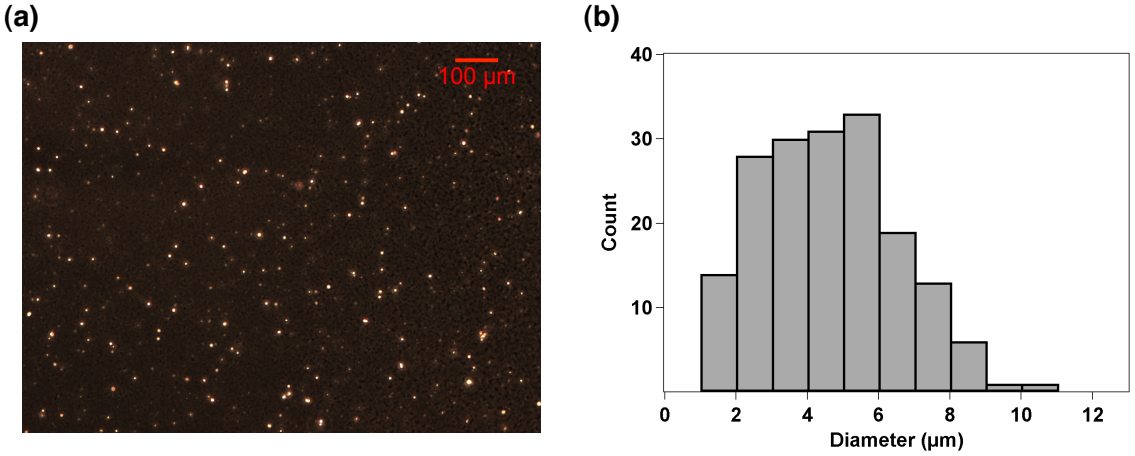

Figure S1. Size analysis of liposome particles. (a) Phase contrast microscope image taken with a Leica D-LUX 3 CCD camera mounted to a Leica DMIL inverted microscope (Leica Microsystems, Wetzlar, Germany). (b) Size distribution of liposomes was characterized by ImageJ software from microscope images. The average diameter of 176 particles was 4.6 ± 2 µm.

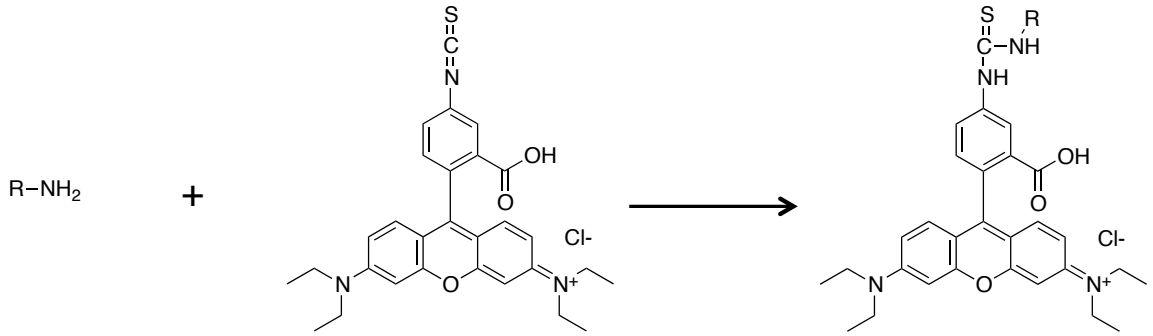

Figure S2. Fluorescent labeling of modified chitosan with rhodamine B isothiocyanate. *R* represents the chitosan backbone. The reaction took place in water at room temperature.

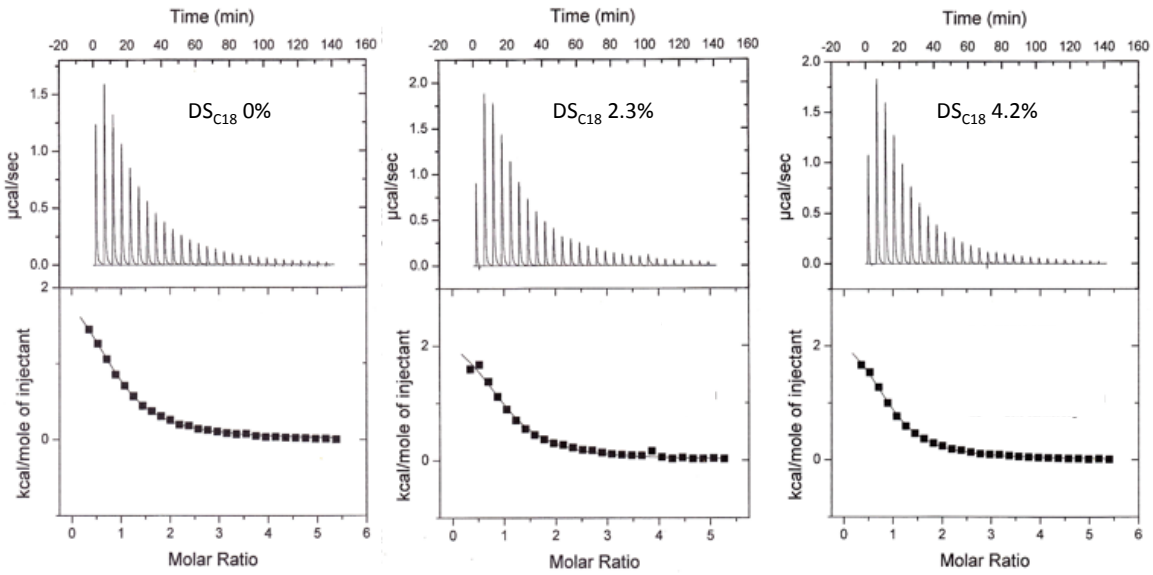

Figure S3. Thermograms of DTPA−chitosan (left), and DTPA−chitosan-g-C_{18} with DS_{C18} = 2.3% (middle) and 4.2% (right). The top row represents raw heat flow data, while the bottom row is data fit to a single-site binding model.

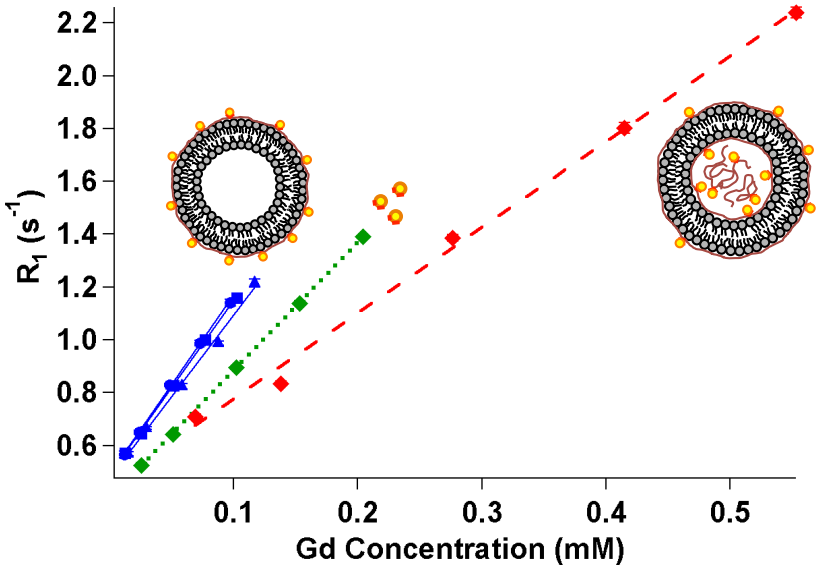

Figure S4. Relaxivity plot for Gd-containing liposome particles. Relaxivities shown in Figure 4 of the text are determined from the slopes of the blue solid curves. The red dashed curve was used to determine the molar relaxivity of DTPA−chitosan-g-C_{18} encapsulated and adsorbed to the liposome (Figure 5). The green dotted curve represents unmodified DTPA−Gd.

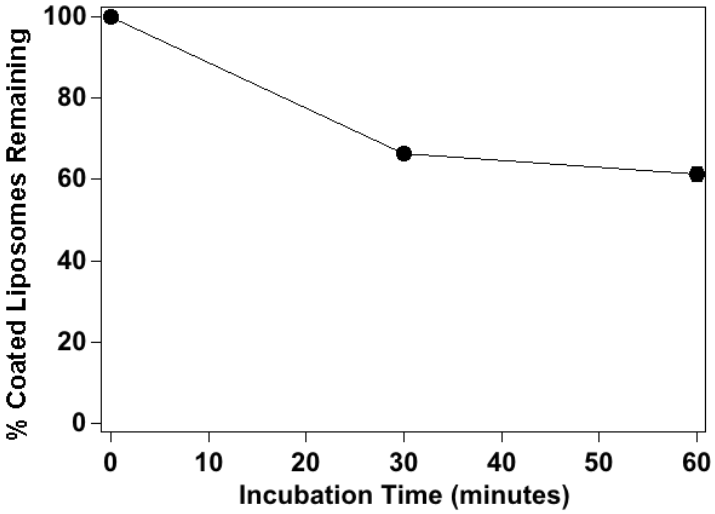

Figure S5. Analysis of association between DTPA−chitosan-g-C_{18} and liposomes in the presence of human serum. Liposomes coated with rhodamine-labeled DTPA−chitosan-g-C_{18} with DS_{C18} = 4.2% were incubated in PBS supplemented with 10% human serum at 37 °C. At each time point, liposomes were centrifuged at 4000 rpm for 10 min and resuspended to determine the amount of modified chitosan remaining on the liposome surface. Data is shown as the average of two replicates per time point, with error bars obscured by data point markers.

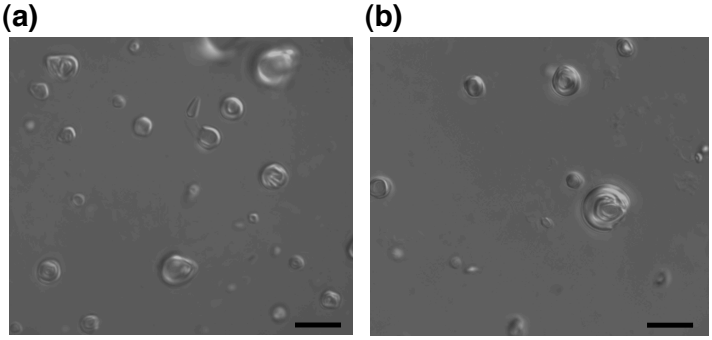

Figure S6. Microscopic images of liposomes coated by DTPA−chitosan-g-C_{18} (DS_{C18} = 4.2%). Images were captured with a Zeiss Axiovert 200M microscope (Carl Zeiss, Oberkochen, Germany) before (a) and after (b) 1 h incubation in PBS supplemented with 10% serum. Scale bars represent 10 μm. The average diameters of liposomes before and after one-hour incubation in serum-supplemented PBS were 4.3 ± 2 and 3.7 ± 2 μm respectively. 272 and 175 particles were measured for the conditions.

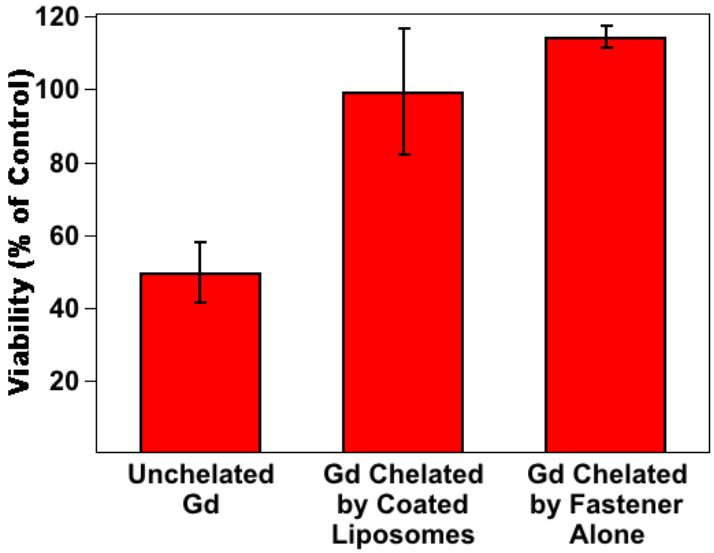

Figure S7. Cellular viability analyzed with an MTT assay for cellular metabolic activity. C166 endothelial cells were seeded on a 96-well plate at $5x10^3$ cells per well. Cells were incubated for 24 h with unchelated gadolinium, or with gadolinium chelated by DTPA−chitosan-g-C_{18} (DS_{C18} = 4.2%) that was either adsorbed to liposomes or free in solution. In each case, gadolinium was kept at a 100 μM concentration, matching that of the highest level used for relaxivity determination of coated liposomes (blue curves in Figure S4). MTT reagent ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ATCC) was added to the cell culture media, and its reduction to formazan dye by metabolically active cells was evaluated by measuring absorbance at 570 nm (Tecan Infinite 200 PRO, Tecan AG, Switzerland). The level of viability was quantified by normalizing the absorbance to that of cells cultured without addition of gadolinium. Error bars represent standard deviation of three replicates.

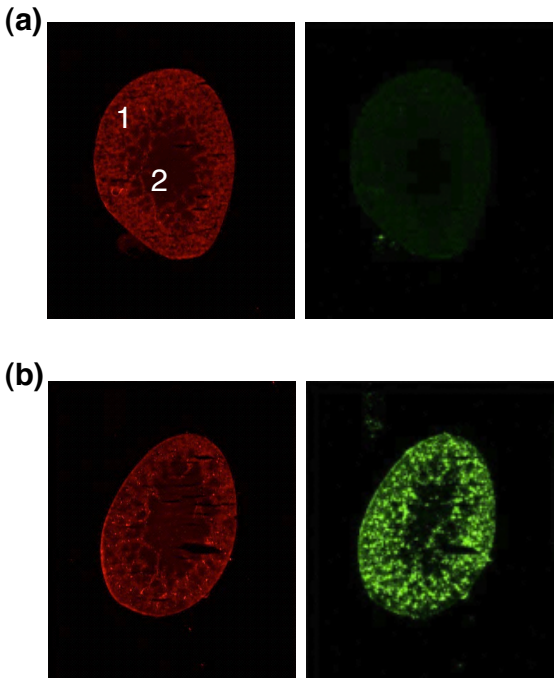

Figure S8. Tissue sections of Sprague Dawley rat kidneys injected with (a) saline, and (b) liposomes labeled with CellVue NIR815 dye. Labeled liposomes were formed by hydration of a lipid film with a solution of NIR815 dye, followed by removal of unincorporated NIR815 by centrifugation. Imaging of frozen tissue sections cut at 30 μm was performed with a LI-COR Odyssey scanner (LI-COR Biosciences, Lincoln, NE). The 680 excitation channel is shown in red and displays tissue autofluorescence. Cortex (marked as **1**) is easily distinguished from medulla (marked as **2**). The 780 excitation channel, shown in green, demonstrates localization of fluorescently labeled liposomes within the cortex.